



Introduction

Early accurate detection and identification of sexually transmitted pathogens is critical for determining proper antibiotic treatment for sexually transmitted infections (STIs) in high risk sexually active individuals.

Table 1. ID/AMR targets detected by the BioCode® STI + Resistance Panel.

Table with 2 columns: STI Organisms (Chlamydia trachomatis, Neisseria gonorrhoeae) and Assay Controls (Endogenous Internal Control, Assay Process Control).

Methods

- Samples for all studies were extracted via the Thermo Scientific KingFisher™ Purification System prior to testing with the BioCode® MDx-3000 in User Defined Mode. Preliminary sensitivity was established by initially testing four 10-fold serial dilutions (contrived in negative urine matrix) with 4 replicates each (2 extractions x 2 extractions).

BioCode® STI + Resistance Panel Workflow

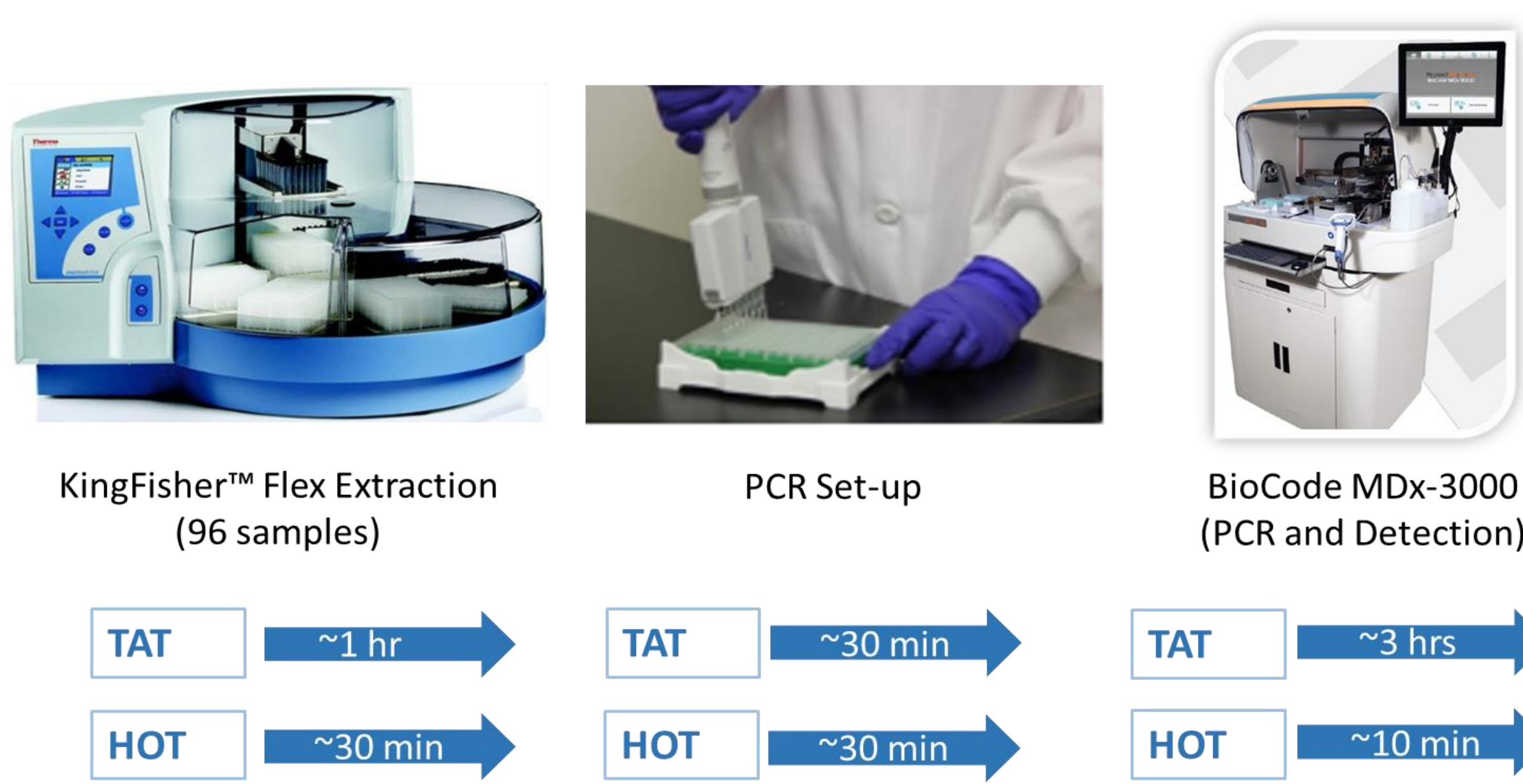


Figure 1. Workflow for BioCode® STI + Resistance Panel. Samples were extracted on the Thermo Scientific KingFisher™ Flex Purification System followed by manual PCR plate setup, then automated PCR and detection on the BioCode® MDx-3000 system.

\*Corresponding author: bladerman@apbiocode.com

Multiplex Detection Targets

The BioCode® STI + Resistance assay consists of 2 ID probes/STI target and 1 probe each for all AMR mutations, NG S91 WT, EIC and APC. Note: MDx-3000 software has masking capabilities to assign custom testing requirements per test sample.

Table 2. BioCode® STI + Resistance Panel detection probes.

Table with columns for Probe, ID Plex, EIC, APC, and various Antibiotic Resistance targets (MG A2058C, MG A2059C, MG S831, NG S91F).

Preliminary Sensitivity

Preliminary sensitivity (eLoD) for each strain was determined by testing 2 extractions x 2 replicates (n=4) at four 10-fold serial dilutions. Strains that had all replicates detected at the lowest concentration were tested at lower concentrations until dropouts were observed.

Table 3. Preliminary sensitivity (estimated LoD).

Table with columns: LoD Strain, Source, and eLoD.

Analytical Reactivity

All strains listed in Table 4 were detected in both replicates (one extraction) by the assay. Detection was observed at ≥10X above the eLoD concentration of the LoD strain associated with weaker sensitivity.

Table 4. Analytical reactivity strains.

Table with columns: Strain Name, Concentration, and Source.

Note: The abstract has been revised since submission.

Cross-Reactivity

No cross-reactivity was observed for the closely-related species tested on the bench (Table 5) nor for the other organisms (via in silico analysis) that may be present in the same anatomical area (Table 6).

Table 5. Closely-related cross-reactivity organisms tested.

Table with columns: Organism, Cross-Reactivity Organism (n=18), Source, and Concentration.

Table 6. Cross-reactivity organisms evaluated via in silico analysis.

Table with columns: Cross-Reactivity Organisms Evaluated via in Silico Analysis (n=179) and various organism names.

Orange = Virus, Yellow = Protozoan

Competitive Inhibition

Competitive inhibition (CI) was not observed for CT, NG, TV or MG when each target was tested at medium concentration (10X eLoD) in the presence of the other 3 STI targets at high concentrations when contrived together in negative urine.

Table 7. Competitive inhibition combinations.

Table with columns: CI Pool, Organism, Level, Final Concentration, and various organism/level combinations.

Antimicrobial Resistance Detection

The BioCode® STI + Resistance assay was able to accurately detect MG or NG AMR mutations in clinical urine samples (Table 8a; previously characterized and/or confirmed by DNA bi-directional sequencing).

Table 8a. Clinical urine samples.

Table with columns: Sample, Genotype, and Concentration.

Table 8b. NG reference strains.

Table with columns: NG Strain, Concentration, and Genotype.

CUS = Clinical urine sample positive for AMR (confirmed by DNA bi-directional sequencing)

Table 8c. Synthetic gene targets.

Table with columns: Synthetic Gene, Concentration, and Genotype.

Conclusions

- The BioCode® STI + Resistance Panel is a highly multiplexed PCR-based assay capable of simultaneously detecting CT, MG, NG and TV as well as mutations associated with antimicrobial resistance for MG and NG. Up to 96 samples can be simultaneously tested per run.