

Rapid 15-minute test to identify the 3 most common gram-negative uropathogens in Urinary Tract Infections

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Abstract

6.2 million cases of Urinary Tract Infection (UTI) are reported in England and Wales every year and have the second highest antibiotic prescription rate. The absence of fast and sensitive diagnostics tests has increased empirical antibiotic use and has contributed to the rise of antibiotic-resistant infections.

To address this challenge, we have developed NANOPLEX®: a rapid 15-minute

test that uses glycan-functionalised-latex nanoparticles, microscopy and image-analysis software to identify and enumerate samples with bacteria.

In this work, we show that NANOPLEX® can correctly identify the 3 most common uropathogens using clinical isolates.

Results

Clinical Strains

79 Clinical strains of E. coli, K. pneumoniae and P. mirabilis were made up in tryptic soy broth (TSB), ran with NANOPLEX® and processed using in-house software.

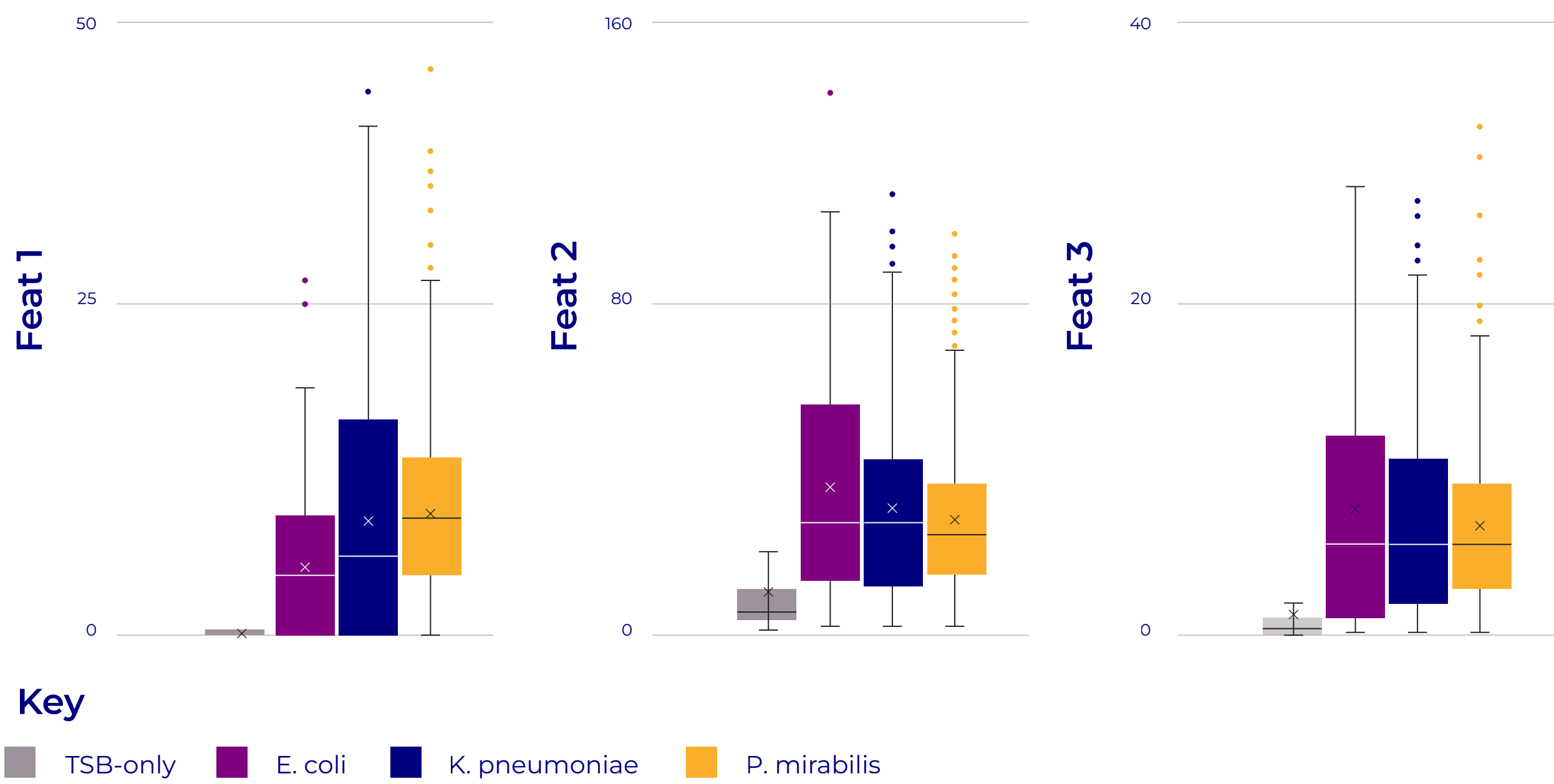


Fig.1: Agglutination of clinical strains of E. coli, K. pneumoniae and TSB-only samples

Clear distinction was seen between positive and negative samples in agglutination features 1, 2 and 3 giving promise that a predictive model could be made (Fig.2)

Model Building

A concentration range of E. coli, K. pneumoniae and P. mirabilis reference strains (10⁴ - 10⁸ CFU/ml) were made up in TSB and run using NANOPLEX® (n= *510).

Agglutination features 1-4 were plotted against each other. Clear distinction between log changes of each bacterial species were observed (Fig.3). This data was used to train XGboost models for each bacterial species.

The positive vs negative prediction model demonstrated specificity, sensitivity and accuracy scores ≥90% for E. coli, K. pneumoniae and P. mirabilis.

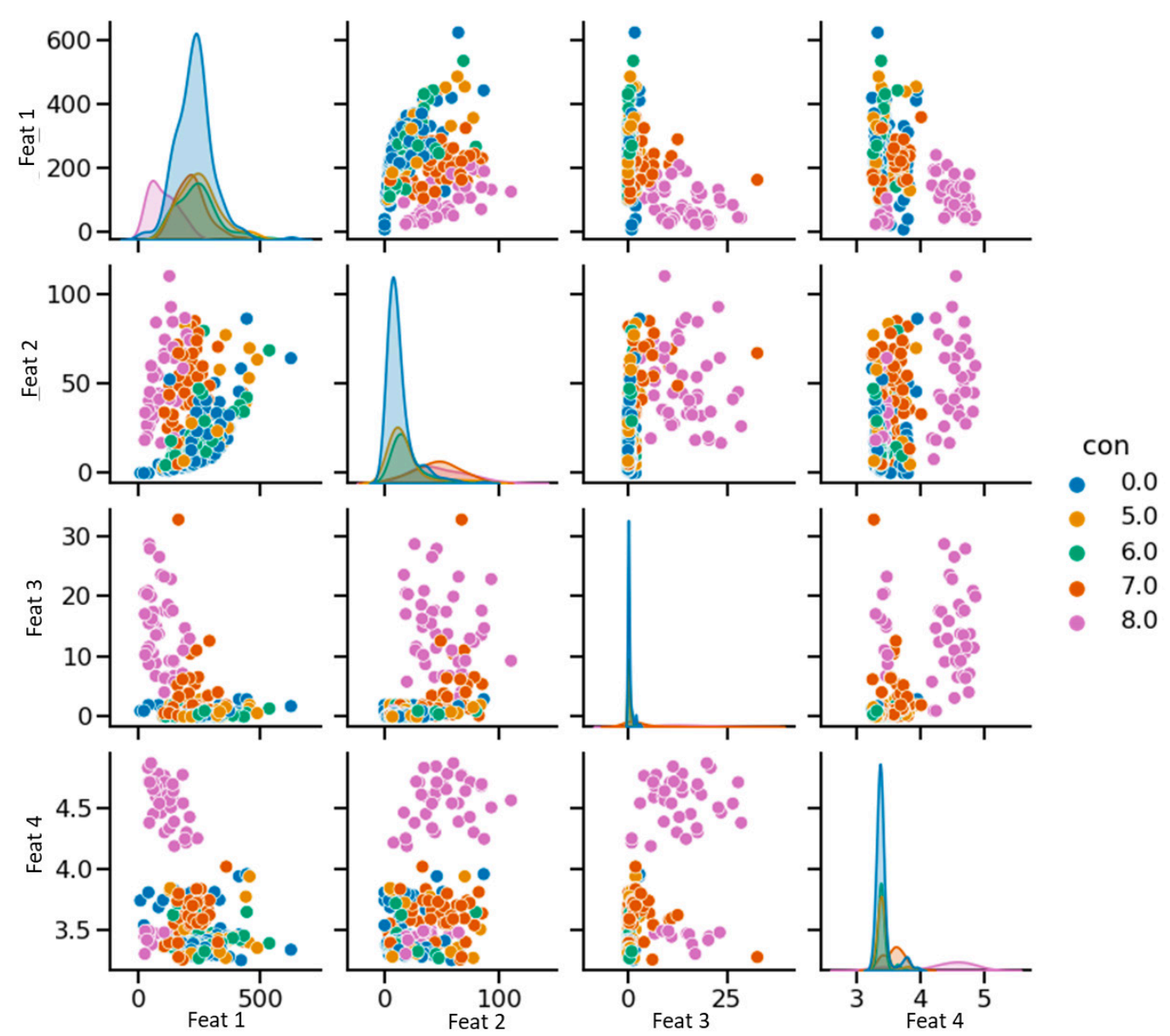


Fig.2: E. coli training data. Agglutination features 1 - 4 were plotted against each other. Distinct populations for each log change in bacteria was demonstrated (10⁴ - 10⁸ CFU/ml) as well as between negative samples.

Blind tests in Synthetic Urine

Clinical strains of E. coli and K. pneumoniae were suspended in synthetic urine (10⁴—10⁸ CFU/ml). Samples were randomised and NANOPLEX® was performed alongside synthetic urine negative controls (n= 165).

The in-house image processing software revealed comparable agglutination signals between training data used to build the models and blind test samples (Fig. 4).

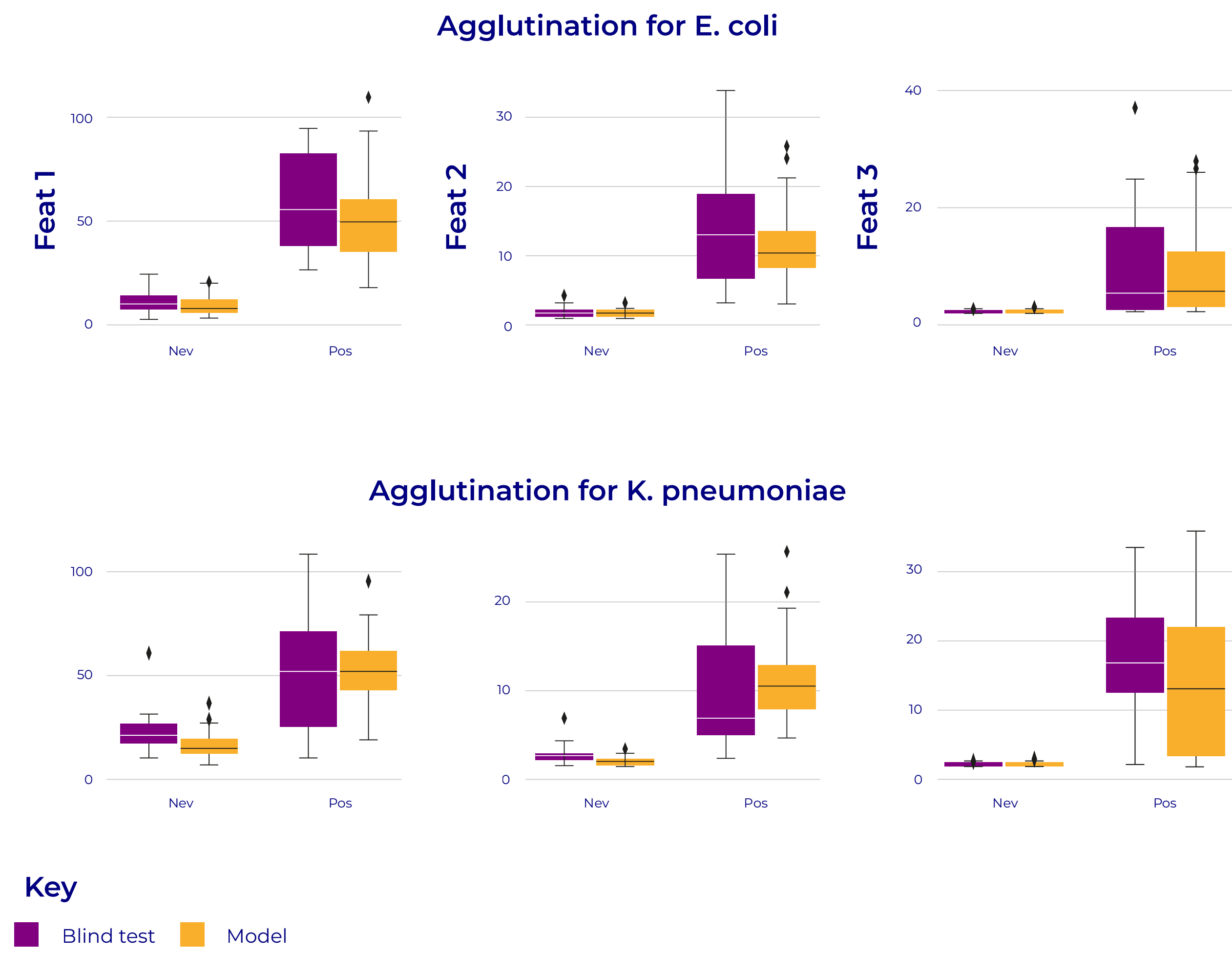


Fig.3: Difference in agglutination between training data used to make the predictive model and blind tests run in synthetic urine.

Specificity, sensitivity and accuracy scores slightly reduced when blind test data was put through the predictive models.

Species	Sensitivity		Specificity		Accuracy	
	Model	Blind test	Model	Blind test	Model	Blind test
E. coli	97% (± 0.03)	91%	99% (± 0.02)	89%	98% (± 0.01)	90%
K. pneumoniae	94% (± 0.05)	87%	99% (± 0.03)	83%	97% (± 0.02)	84%
P. mirabilis	90% (± 0.08)	-	93% (± 0.08)	-	91% (± 0.05)	-

Method: The NANOPLEX® assay

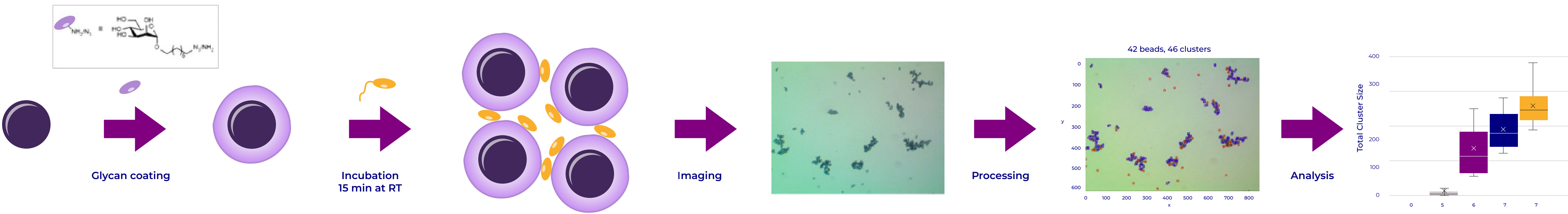


Fig 4. Latex microspheres functionalised with glycans using EDC or copper-catalysed azide-alkyne cycloaddition were incubated with clinical isolates of E. coli and K. pneumoniae. After a 15 minute incubation period, samples were imaged by bright field microscopy and analysed by FluoretiQ's in-house image processing software [11].

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