QIAxcel® Advanced System: Applications In A Small-Scale Veterinary Diagnostic Laboratory

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Veterinary Medicine Today: Role and Challenges

Veterinary medicine is not merely concerned with the treatment of animals. It also plays a role in the prevention of disease outbreak and transmission and is a key element in ensuring public safety, through protection of public health and the environment.

Today, a significant challenge is in finding a rapid and accurate method for identification of pathogens. Even though great advances have been made in the field of molecular biology over the last decade, conventional PCR is still considered a basic and reliable tool in molecular diagnostics.

Here, we demonstrate how the QIAxcel Advanced System provides fast and accurate results, thereby enabling reliable identification of a range of important pathogens.

Main areas of interest in the Laboratory for Molecular Bacteriology of Veterinary Faculty Ljubljana

Veterinary pathogens	Fish, shellfish, crayfish pathogens	Bee pathogens	Emerging zoonotic pathogens	Foodborne pathogens	Bioterrorism agents
Mycobacterium avium	*Aphanomyces astaci*	*Nosema* spp.	MRSA	*Escherichia coli*	*Bacillus anthracis*
Clostridium perfringens	*Aeromonas salmonicida*	*Paenibacillus larvae*	*Clostridium difficile*	*Listeria monocytogenes*	*Coxiella burnetii*
Campylobacter spp.	*Bonamia* spp.			Thermotolerant campylobacters	*Francisella tularensis*
Taylorella equigenitalis	*Marteilia refringens*			*Salmonella* spp.	*Vibrio cholerae*
Lawsonia intracellularis	*Mycobacterium* spp.				*Yersinia pestis*
Enterococcus spp.	*Renibacterium salmoninarum*				*Burkholderia mallei*
Brachyspira hyodysenteriae	*Tetracapsuloides bryosalmonae*				
Anaplasma phagocytophilum					
Borrelia spp.					

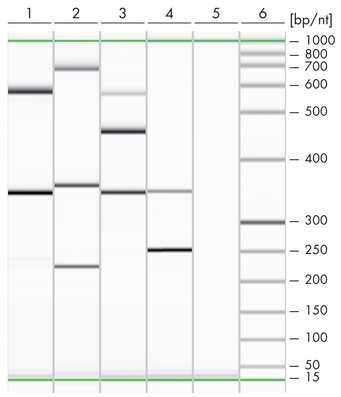
Detection of *Clostridium perfringens*

C. perfringens is widely distributed in the environment and in the gut of animals and humans. It is classified into types A, B, C, D and E on the basis of 4 major toxins.

In humans, it causes food poisoning. While in animals, *C. perfringens* causes necrotic enteritis in broilers, leading to high mortality and production losses.

Using multiplex PCR and QIAxcel Advanced, genes *cpa, cpb, etx, iA, cpb2* and *cpe,* encoding the toxins, can readily be detected.

Culture of *Clostridium perfringens* on sheep blood agar.
(Photo courtesy of J. Avberšek)

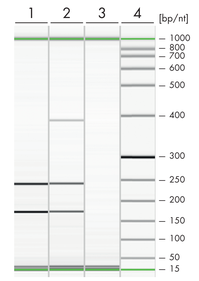


Identification of toxin-encoding genes using multiplex PCR and QIAxcel Advanced System. The QIAxcel DNA Screening Kit and AM420 method were used. QIAxcel alignment markers 15 bp–1 kb and DNA size marker 50–800 bp were run simultaneously. **Lane 1:** *cpb2, cpa;* **Lane 2:** *etx, cpa, cpb;* **Lane 3:** *cpb2, iA, cpa;* **Lane 4:** *cpa, cpe;* **Lane 5:** NTC; **Lane 6:** DNA size marker (50–800 bp).

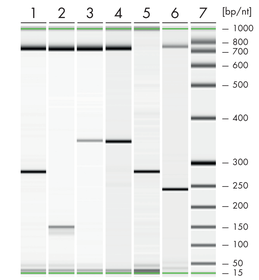
Identification of *C. difficile* and *Campylobacter* spp.

Clostridium difficile is an important cause of hospital-acquired diarrhea in humans. The frequency and severity of community-acquired *C. difficile*-associated disease has increased. Animals may serve as reservoir for the pathogen, whose zoonotic potential has yet to be proven.

Thermotolerant campylobacters are the causative agents of intestinal campylobacteriosis, the leading bacterial, foodborne zoonosis worldwide. Due to their fastidious growth requirements, they are difficult to detect and identify with traditional methods, but QIAxcel-based methods renders it possible.



PCR product analysis of *Clostridium difficile* strains on QIAxcel Advanced. Lane 1: *tcdB* and *tpi;* **Lane 2:** *tcdB, tpi* and *tcdA;* **Lane 3:** NTC; **Lane 4:** DNA size marker 50–800 bp.

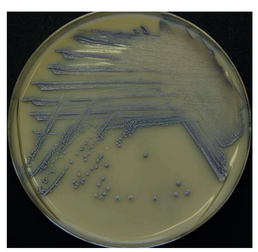


PCR product analysis of thermotolerant campylobacters on QIAxcel Advanced. Lanes 1 and 5: *C. lari;* Lane 2: *C. coli;* **Lanes 3 and 4:** *C. jejuni;* **Lane 6:** *C. upsaliensis;* **Lane 7:** DNA size marker 50–800 bp. Amplicon of the 23S rRNA gene amplified as an internal control is visible on all lanes with the exception of lane 5.

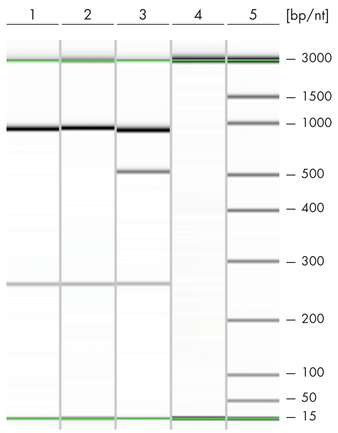
Identification of Methicillin-Resistant *S. aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is not only a human problem — it also affects livestock, potentially serving as a reservoir of resistant strains. In order to efficiently manage MRSA infections, there is a need for a method that enables rapid detection and accurate identification.

Here, we have used an experimental setup based on conventional multiplex PCR coupled with QIAxcel analysis. It shows that the method may prove useful in addressing the problem of efficient MRSA surveillance.



MRSA culture. (Photo courtesy of M. Lepen)

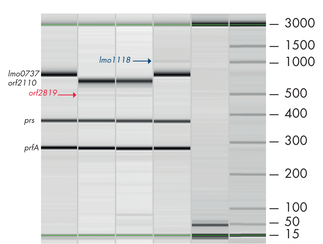


Gel image demonstrating detection of 16S rDNA, *nuc* and *mecA* genes using QIAxcel Advanced. Lanes 1, 2: *S. aureus* (*nuc+*); **Lane 3:** MRSA (*nuc+, mecA+*); **Lane 4:** NTC; **Lane 5:** DNA size marker.

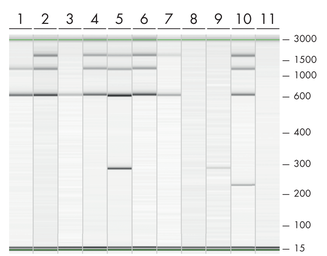
Characterization of *L. monocytogenes* and *Brucella* spp.

Listeria monocytogenes is a foodborne pathogen which causes listeriosis in animals and humans and has the highest mortality rate of all foodborne bacterial pathogens. Serotyping is used as a first level of discrimination, but with QIAxcel, *orf2819, orf2110, lmo0737, lmo1118, prs* and *prfA* genes can clearly be detected, supplying critical information for accurate assessing of the PCR-serotypes.

Another threat to human health that can be monitored with the aid of QIAxcel is *Brucella* spp. — a cause of a zoonosis of global importance and a potential bioterrorism agent. Classical detection methods are time-consuming and the homogeneity of the genus makes identification to the species level difficult. Our method enables differentiation between different *Brucella* species.



QIAxcel ScreenGel image demonstrating the capacity of the system to identify genes expressed by *Listeria monocytogenes*.



Differentiation of *Brucella* species. Lane 1: *B. ovis;* **Lanes 2, 4, 6:** *B. melitensis;* **Lanes 3, 9:** Non-identified; **Lane 5:** *B. suis;* **Lane 7:** *B. abortus;* **Lane 8:** Negative sample; **Lane 10:** Positive control; **Lane 11:** NTC.

Conclusions

- Fast and reliable diagnostics in veterinary microbiology plays an important role in the battle against animal infectious diseases. It is therefore of great clinical interest to define an approach that allows rapid and efficient identification of pathogens, in order to implement the appropriate measures for the benefit of animal and public health.
- The QIAxcel Advanced System was introduced for separation and analysis of single or multiplex PCR products to improve the quality of the analysis and traceability, and also to reduce testing time.
- Based on the data of ~600 runs over the last 3 years, we conclude that the QIAxcel Advanced System significantly minimizes manual intervention, increases work safety and provides better traceability of the data.
- The associated ScreenGel software reduces turnaround time by automated analysis and identification of pathogens.
- Therefore, the QIAxcel Advanced System makes an important contribution to improved veterinary diagnostics.

For more information, please contact **mirjana.kozulic@qiagen.com**

The applications presented here are for research use only. Not for use in diagnostic procedures.

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Sample to Insight