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# Validation of a microbiological inhibition system based on Eclipse Farm 3G coupled with e-Reader to screening β-lactam and tetracycline antibiotics in goat's cheese whey

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**Abstract.** The presence of antimicrobial residues in milk and dairy products could cause negative technological effects and represents a risk for consumer health. During the cheese-making process, antibiotics could be retained in curd or eliminated in whey to a greater or lesser extent. Whey is a by-product used in the manufacture of foodstuffs for human consumption and animal feeding and therefore, the presence of antibiotic residues in this product should be controlled. Thus, it is crucial to develop an analytical strategy to screen antibiotics in whey. A new system for screening antibiotics in raw milk was recently developed, in which a microbial inhibitor tube test (Eclipse Farm) and a device (e-Reader) are coupled. The new system allows the incubation of the test and a continuous monitoring of the test colour change. The aim of this work was to study the performance of the new system for the detection of β-lactam and tetracycline residues in goat's cheese whey. A preliminary study demonstrated the necessity to include a one-hour diffusion period at room temperature. The performance was validated in agreement with European Commission Decision 2002/657/EC. Specificity of microbial test was evaluated by analysing one hundred and thirty whey samples. Results showed a low percentage of false-positive results. The detection limits for amoxicillin, benzilpenicillin, cephalexin and oxytetracycline in goat's cheese whey were calculated and the detection capabilities (CCβ) were below or at the MRL levels, with the exception of amoxicillin, which was slightly above them. In conclusion, Eclipse Farm coupled to e-Reader represents an appropriate method to screen β-lactams and tetracycline residues in whey.

**Keywords.** Whey – Antibiotic – β-lactams – Tetracyclines – Screening test.

***Validation d'un système d'inhibition microbiologique basé sur Eclipse Farm 3G couplé avec un lecteur e-Reader pour le dépistage des antibiotiques β-lactam et tetracycline dans le lactosérum de fromage de chèvre***

***Résumé.*** *La présence de résidus d'antibiotiques dans le lait et les produits laitiers peut entraîner des problèmes technologiques et un risque pour la santé des consommateurs. Pendant le processus de fabrication du fromage, les antibiotiques peuvent rester attachés au caillé ou peuvent être éliminés dans le sérum. Le lactosérum est un sous-produit utilisé dans la fabrication de denrées alimentaires destinées à la consommation humaine et à l'alimentation animale et, par conséquent, la présence de résidus d'antibiotiques dans ce produit doit être bien contrôlée. Il est donc essentiel de mettre en place une stratégie analytique pour l'analyse des antibiotiques dans le lactosérum. Un nouveau test d'inhibition microbienne (Eclipse Farm) couplé avec un dispositif d'incubation et de lecture (e-Reader) a été récemment développé pour le dépistage d'antibiotiques dans le lait cru. Le nouveau système permet l'incubation et la surveillance continue du changement de la couleur du test. L'objectif de ce travail consistait à étudier la performance du nouveau système pour la détection des résidus de β-lactames et de tétracyclines dans le lactosérum du fromage de chèvre. Une étude préliminaire a démontré la nécessité d'inclure une étape de diffusion d'une heure à température ambiante. Le système a été validé conformément aux procédures décrites dans la décision 2002/657/CE de la Commission Européenne. La spécificité du test a été évaluée en analysant 130 échantillons de lactosérum. Les résultats ont montré un faible pourcentage de résultats faussement positifs. Les limites de détection pour l'amoxicilline, la benzilpenicilline, la cé-*

*phalexine et l'oxytétracycline dans le lactosérum du fromage de chèvre ont été calculées, ainsi que les capacités de détection (CCβ), toutes en dessous ou au même niveau que les Limites de Résidus Maximum (LRM), sauf pour l'amoxicilline, avec une limite légèrement supérieure. En conclusion, Eclipse Farm couplé à e-Reader représente une méthode appropriée pour la détection des résidus des β-lactames et tétracyclines dans le lactosérum de chèvre.*

***Mots-clés.*** *Lactosérum – Antibiotique – β-lactames – Tétracyclines – Test de dépitage.*

## I – Introduction

The wide use of antimicrobials in food-producing animals, to prevent and to treat diseases, can constitute a problem in milk and dairy products due to the presence of antibiotic residues. In fact, their presence in milk could cause negative technological effects (Berruga *et al*., 2008). Moreover the presence of antibiotics in milk products could have direct health implications for the consumers with allergic reactions and lead to an increase of resistant bacteria with serious consequences in human and animal health (WHO, 2014). To guarantee the consumer health, the European Union established the Maximum Residue Limits (MRL) in milk but up to now, no limits has been established for dairy products, as cheese and whey (EC, 2010).

Whey is a by-product of cheese-making process with many applications in food industry and livestock and therefore, possible presence of drug residues could have direct negative effects for human and animal health and in dairy sector by affecting fermentation processes. During cheese-making process, antibiotics present in milk, could be retained in cheese or carried in whey depending of the chemical nature of each substance (Cabizza *et al*., 2016). For all these reasons, the availability of an analytical strategy to screen antibiotics in whey seems necessary.

In last years the strategy to control of antibiotics in milk has been introduced at different levels of the chain. Nowadays, not only central laboratories but dairies and farmers are aware with the control of antibiotics. Thus, the availability of automatic screening methods has become a real need. A new system for the screening of antibiotics in raw milk was recently developed (Mata *et al*., 2016). This new system combine a microbial inhibitor tube test (Eclipse Farm 3G) and a device (e-Reader) that allows the incubation of the test and the continuous monitoring of the color change. The aim of this work was to study the performance of this new system for detection of β-lactams and tetracyclines in goat's cheese whey.

## II – Material and methods

### 1. Whey Samples

Whey samples were produced by the application of a laboratory scale cheese-making, as described by Giraldo *et al.* (2017). Raw milk was obtained from the experimental flock of healthy and untreated Murciano-Granadina goats at Universitat Politècnica de Valencia (Valencia, Spain). Whey samples were analyzed for chemical composition using MilkoScan 6000 (Foss, Hillerød, Denmark) and the pH value was valued by a pH-meter (Crison, Barcelona, Spain).

### 2. Antibiotics

Four antibiotics representative of the most used molecules in veterinary were chosen. The molecules tested were amoxicillin, benzilpenicillin, cephalexin, and oxytetracycline. All antibiotics were purchased from Sigma-Aldrich Química, S.A. (Madrid, Spain). Stock solutions (1 mg/mL) were prepared in according to International Dairy Federation (ISO/IDF, 2003). Working dilutions were prepared daily.

### 3. Performance of Eclipse Farm 3G Test and e-Reader

To assay the detections of antibiotics in whey, Eclipse Farm 3G test (ZEULAB, Zaragoza, Spain) coupled to the e-Reader detector were used. The test procedure was carried out following manufacturer recommendations, doing a preliminary study to improve the system for the analysis of milk whey samples. This modification of original instructions was necessary to eliminate gradients of color (purple and yellow). For this, 10 goat's milk whey samples were tested with different treatments: centrifugation (3000 rpm, 10 minutes), heating (80ºC, 10 minutes), centrifugation and heating (under the same conditions) and with pre-diffusion for one hour at room temperature.

Previously, cut-off level for Eclipse Farm coupled to e-Reader was calculated following indications by Mata *et al.* (2016). Seventy-five goat's milk whey samples were used, obtaining their mean and standard deviation. The cut-off for microbiological test was the result of mean value plus three times the standard deviation.

A study of false positive rate was also carried out and, in this case, 130 negative goat's milk whey samples were tested.

To determinate the detection capabilities (CCβ) with whey samples the method previously described by Mata *et al.* (2016) was applied according to the CRL guidelines (CRLs, 2010).

## III – Results and discussion

After evaluating several procedures to prepare samples for the analysis, adding a step of pre-diffusion for 1 hour at room temperature was selected as the best choice.

The cut-off was calculated by using the mean value from 75 negative samples (41.2) and the corresponding standard deviation (7.0). The result was of 62.1 (mean of the negative samples plus 3 times the standard deviation), as it is observed in Fig. 1. Thus, e-reader values equal or above 62 were considered as positive samples.

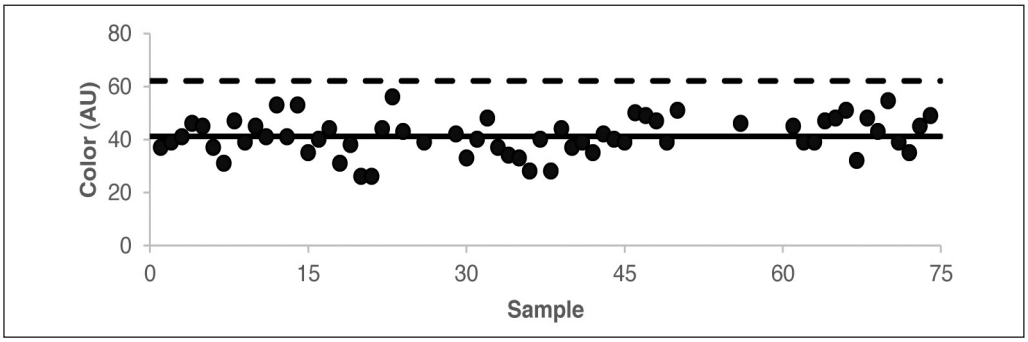


**Fig. 1. Results obtained by e-Reader for 75 goat's whey samples analysed with Eclipse Farm test. Solid line indicates the average value. Dashed line indicates the proposed cut-off level with a confidence interval higher than 99%.**

The composition of goat's whey samples is presented on the Table 1. A minimum false-positive rate was found for these goat's milk whey samples, which was equal to 2.3%. This percentage is in agreement with the false positive rate calculated by Beltrán *et al.* (2015) for goat's milk using different microbial inhibitor tests (BRT MRL, 1.4%; Delvotest SP-NT, 4.3%; Delvotest DA, 3.1%; Eclipse 100, 0.6%). Therefore, Eclipse Farm coupled to e-Reader continues being appropriated, on terms of false-positive results, when whey matrix is analyzed.

**Table 1. Quality parameters of goat's milk whey samples free of antimicrobials**

| Parameters | Mean | SD[a] | Minimum | Maximum |
|---|---|---|---|---|
| pH | 6.45 | 0.22 | 5.46 | 6.69 |
| Fat | 0.68 | 0.16 | 0.33 | 1.21 |
| Protein | 1.09 | 0.15 | 0.83 | 1.48 |
| Lactose | 4.91 | 0.10 | 4.63 | 5.10 |
| Total solids | 6.69 | 0.16 | 6.36 | 7.12 |

[a] SD: standard deviation.

The detection limits and detection capabilities goat's milk whey for 4 antimicrobials are summarized in Table 2.

**Table 2. Detection limits (µg/kg) and detection capabilities of Eclipse Farm coupled to e-Reader for four representative β-lactams and tetracycline residues in goat's milk whey**

| Antimicrobial | MRL[a] (µg/kg) | Detection limit[b] (µg/kg) | e-Reader value[c] | Detection capability[b] (µg/kg) | No. positive[d]/ no. samples | e-Reader value[d] |
|---|---|---|---|---|---|---|
| Amoxicillin | 4 | 4 | 71.4 ± 5.3 | 5 | 20/20 | 106.8 ± 11.8 |
| Benzilpenicillin | 4 | 3 | 107.0 ± 15.2 | 3 | 40/40 | 117.2 ± 24.4 |
| Cephalexin | 100 | >50 | 63.2 ± 18.4 | 60 | 39/40 | 108.4 ± 19.2 |
| Oxytetracycline | 100 | >60 | 67.8 ± 12.3 | 100 | 60/60 | 94.9 ± 12.2 |

[a] MRL: EU Maximum Residue Limit (µg/kg).

[b] Positive results are defined as an e-Reader value higher than 62.

[c] Mean ± SD (n = 5).

[d] Mean ± SD.

According to the Table 2, all the obtained detection limits are below or equal to their corresponding MRLs. However, in the case of detection capabilities, in which a higher number of samples is tested, amoxicillin has shown a higher value (CCβ = 5 µg/kg) than their legal limit. It is observed that when a low e-Reader value is determined for the detection limit (amoxicillin and oxytetracycline) the corresponding CCβs are higher but with high e-Reader values (Table 2). These results suggest that CCβs for both molecules are close to detection limits but statistically do not reach the same level. Moreover, standard deviations were relatively high, suggesting that there is an important variability in the results between repetitions of fortified whey samples.

Comparing our results with those reported by other authors in milk, some differences are found. Beltrán *et al.* (2015) obtained CCβ higher than the calculated in this study using different microbiological tests (BRT MRL, Delvotest SP-NT, Delvotest DA and Eclipse 100) in sheep and goat's milk. In the case of goat's milk, in general, all detection capabilities were lower than the obtained for cheese whey samples, with the exception of the cephalexin, whose CCβ for BRT MLR and Delvotest SP-NT was around or above their MRL (>100 µg/kg and 75 µg/kg respectively) and oxytetracycline could not be detected at MRL by any test used in this study.

Using Eclipse Farm coupled to e-Reader to analyze cow's milk samples, Mata *et al.* (2016) studied detection limits and detection capabilities for these same antimicrobials and they obtained results too similar to this study. Regarding detection capabilities, the e-Reader values in cow's milk were lower than in goat's cheese whey for amoxicillin (CCβ = 4 µg/kg) and cephalexin (CCβ = 50 µg/kg), but for oxytetracycline, detection capability was the same for both matrices.

Finally, these results could be compared with the obtained by Giraldo *et al.*, (2016), who tested also these same antibiotics in goat's milk whey, using Eclipse 100 (ZEULAB, Spain), a microbiological

test with similar characteristics than the used in this study. Detection capabilities were slightly lower than the calculated in this study for amoxicillin (CCβ = 3.9 µg/kg), benzylpenicillin (CCβ = 2.6 µg/kg) and cephalexin (CCβ = 48.4 µg/kg). On the contrary, for oxytetracycline, the CCβ was above the MRL of this molecule (116.8 µg/kg).

## IV – Conclusions

Eclipse Farm coupled to e-Reader allow to detect benzipenacillin, cephalexin and oxytetracycline to concentrations below or equal to their MRLs, with a minimum rate of false-positive results. Thus, this method could be a good alternative to screening of β-lactamsand tetracyclines residues in cheese whey.


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