



Are antibiotic screening approaches adequate?

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1. Set up of the proficiency test

- This proficiency test focused on the analysis of antibiotics belonging to the aminoglycoside, macrolide, quinolone, β -lactam, sulfonamide and tetracycline group in beef.
- Three beef materials were prepared by spiking and cryogenic mincing:
 - I: blank
 - II: 280 $\mu\text{g}/\text{kg}$ flumequine
 - III: 120 $\mu\text{g}/\text{kg}$ lincomycin and 230 $\mu\text{g}/\text{kg}$ spectinomycin
- Each participant received one sample (75g) of each material.
- Participants were asked to carry out their routine screening analysis and confirm the presence and quantify the amount of the detected antibiotics.
- 26 laboratories participated of which 23 applied a screening test.

2. Results - false negatives and false positives

Of the 23 laboratories only 9 characterized all three samples correctly (compliant/suspect) and only 3 indicated the correct compounds or compound groups. A result was considered as a false negative result if (1) an antibiotic was not detected although it was present in the sample and (2) the laboratory did not indicate that the antibiotic (group) was not included in the method.

| | |
|------------------------------|-----|
| False negative rate overall† | 53% |
| Microbiological methods | 73% |
| Biochemical methods | 50% |
| Instrumental methods | 22% |
| False positive rate | 7% |

† each compound and method considered separately

3. Conclusions

- Most laboratories managed to work with the cryogenically minced beef materials in their screening tests, demonstrating the suitability of spiked muscle material for proficiency tests focusing on the screening analysis.
- Many laboratories improved the EU 4-plate test with supplemental plates. Nonetheless a very high false negative rate is observed.
- The PremiTest gave unreliable results, but a preceding solvent extraction is a potential improvement.
- The Nouws Antibiotic Test is the only microbial test detecting LMC. It fails to detect SMC, but the presence of LMC should trigger additional confirmation of SMC because these two antibiotics are co-administered.
- Targeted LC-MS/MS methods are highly specific and primarily fail if compounds are not included. Establishing a complete set of compounds of interest on forehand is crucial in preventing false negative results.
- LC-ToF/MS methods fail if compounds are not included in the database or if sensitivity for certain compounds is lacking.
- A lot of effort is needed to improve screening approaches.**

AG = aminoglycoside
M = macrolide
Q = quinolone
 β = β -lactam
S = sulphonamide
T = tetracycline

GI = growth inhibitor
Flu = flumequine
LMC = lincomycin
SMC = spectinomycin
-- = no antibiotic detected

Correct
False negative
False positive

Physicochemical and biochemical tests

| Lab | AG | M | Q | β | S | T | Material | | |
|-----|-----------------|---------------------|---------------|------------------------|---------------------|-----------------------|----------|-----|---------|
| | | | | | | | I | II | III |
| 2 | Not tested | LC-MS/MS | Ridascreen | LC-MS/MS | LC-MS/MS | ELISA | - | Q | - |
| 3 | LC-MS/MS | LC-UV | LC-FLD | LC-UV | LC-UV | LC-UV | - | Flu | LMC |
| 9 | LC-MS/MS | | | | | | - | - | - |
| 10 | Charm II strep. | Charm II macrolides | Q EIA Flu EIA | Charm II β -STAR | Charm II sulfa drug | Tetrasensor tissue 20 | - | Flu | GI |
| 13 | LC-MS/MS | | | | | | - | Flu | LMC SMC |
| 14 | | LC-MS | LC-FLD | Charm | Charm | Charm | - | Q | - |
| 17 | Charm II | Charm II | LC-FLD | Charm II | LC-DAD | Charm II | - | Flu | - |
| 18 | Not tested | LC-MS/MS | LC-DAD/FLD | LC-MS/MS | TLC | Charm II | - | - | - |
| 19 | HRLC-ToF/MS | | | | | | - | Flu | SMC |
| 21 | HRLC-ToF/MS | | | | | | - | Flu | - |
| 22 | LC-MS/MS | | | Not tested | | | - | Flu | LMC SPM |
| 24 | LC-MS/MS | | | | | | - | Flu | LMC |
| 25 | LC-MS/MS | | | | | | - | Flu | LMC SMC |

Microbial tests: EU 4-plate test (and derived)

| Lab | AG | M | Q | β | S | T | Material | | |
|-----|-------------------------------------|-------|--------------------|-------------------------------------|-------|----------------------|----------|----|-----|
| | | | | | | | I | II | III |
| 8 | EU4pt | EU4pt | <i>E. coli</i> pH8 | EU4pt | EU4pt | <i>B. cereus</i> pH6 | M | M | - |
| 11 | EU4pt | EU4pt | <i>E. coli</i> pH8 | EU4pt | EU4pt | EU4pt | - | - | - |
| 12 | EU4pt | EU4pt | <i>E. coli</i> pH8 | EU4pt | EU4pt | EU4pt | - | - | - |
| 15 | EU4pt | EU4pt | <i>E. coli</i> pH8 | EU4pt | EU4pt | <i>B. cereus</i> pH6 | B/M | - | - |
| 16 | EU4pt | EU4pt | <i>E. coli</i> pH8 | EU4pt | EU4pt | <i>B. cereus</i> pH6 | - | - | - |
| 20 | <i>B. subtilis</i> pH6, 7.4 and 8 | | | | | | - | - | - |
| 26 | EU4pt + <i>B. stearthermophilus</i> | | <i>E. coli</i> pH8 | EU4pt + <i>B. stearthermophilus</i> | | | - | - | - |

Microbial test: PremiTest

| Lab | AG | M | Q | β | S | T | Material | | |
|-----|--------------------------------|-----------|---|-----------|---|---|----------|----|-----|
| | | | | | | | I | II | III |
| 6 | PremiTest | | | | | | GI | GI | GI |
| 10 | Solvent extraction + PremiTest | | | | | | - | - | GI |
| 22 | PremiTest | | | PremiTest | | | - | - | - |
| 24 | PremiTest | PremiTest | | PremiTest | | | - | - | - |

Microbial tests: miscellaneous

| Lab | AG | M | Q | β | S | T | Material | | |
|-----|------------------------------|--------------------------|-------------------------|----------------------------|-----------------------|---------------------------------------------------------|----------|-----|-----|
| | | | | | | | I | II | III |
| 4# | <i>B. subtilis</i> BGA pH8.5 | <i>K. rhizophila</i> pH8 | <i>Y. ruckeri</i> pH6.5 | <i>K. rhizophila</i> pH8.5 | <i>B. pumilus</i> pH7 | <i>B. cereus</i> pH6 | - | Q | M |
| 7# | <i>B. subtilis</i> BGA pH8.5 | <i>K. rhizophila</i> pH8 | <i>Y. ruckeri</i> pH6.5 | <i>K. rhizophila</i> pH8.5 | <i>B. pumilus</i> pH7 | <i>B. cereus</i> pH6 | - | Q | M |
| 10 | | | <i>E. coli</i> | | | | - | Flu | |
| 14 | <i>B. subtilis</i> | <i>K. rhizophila</i> | | | | <i>B. cereus</i> 1178 <i>B. cereus</i> K250 Charm | - | - | - |

Nouws Antibiotic Test

