Detection of Viable Microorganisms
Using Propidium Monoazide

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Why would we want to know if there are living microorganisms on something?
A congressman is mailed a letter containing a white powder. Does it contain Anthrax?
We also want to avoid false positives when looking for life.

Is a rover carrying Earth-life to other planets?
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Let's say we want to sterilize something - for example, the next Mars rover.
Some sterilization techniques will compromise cell membranes, allowing PMA to enter.
When exposed to light, PMA crosslinks with DNA.
DNA is precipitated out of solution.
Imagine you have a sample:

DNA from viable organisms is still in solution.

DNA from nonviable organisms has been precipitated out.
qPCR

Amplifies the DNA in solution. Quantifiable as to how much DNA you started with.
Only the DNA of viable organisms is amplified.

These are the organisms that sterilization did not kill.
The literature on PMA is conflicting and incomplete.

Ourselves and others have found that:

In a fully sterilized sample...

A small fraction of the DNA is not precipitated.

Yet, there is no amplification.
For DNA to be amplified during qPCR, DNA polymerase, an enzyme, must be able to bind to the template DNA and process.

Could PMA inhibit this function, in addition to causing precipitation?
When doing qPCR, you select how long of a piece of DNA you want the polymerase to amplify.

Length influences qPCR’s determination of viability, therefore a mechanism other than precipitation must be occurring.
Statistically, PMA is more likely to have a binding spot on a longer piece of DNA.
When doing qPCR, you select how long of a piece of DNA you want the polymerase to amplify.

Length influences qPCR’s determination of viability, therefore a mechanism other than precipitation must be occurring.
**Exonucleases**

Exonucleases are enzymes that degrade DNA from the ends. Could PMA inhibit this function?

[Diagram of DNA and exonuclease action]

PMA inhibits exonuclease function.

**Endonucleases**

Endonucleases are enzymes that cut DNA at specific sequences. Could PMA inhibit this function?

[Diagram of DNA and endonuclease action]

PMA does not seem to inhibit endonuclease function.

Three endonucleases were mixed with E. coli DNA and run on a gel.

[Image of gel with bands]

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The Next Step

This would give us a better idea of how PMA blocks DNA amplification.
We can use already available bioinformatics data to look at shared binding sites.
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Conclusion

In addition to precipitating DNA out of solution, PMA can inhibit the function of some enzymes.
Wait. Remind me why this is important.

Understanding how PMA works will tell us about its limits of detection.

Under what conditions will PMA do what we want it to do?

If the conditions are something we can work with: Is that a bio-threat? Is the rover sterilized?
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